6/23 Chitosan Nanoparticle Formation trial 1

2016年6月27日　　下午 06:10

3 mg/mL chitosan
    Dissolve with 1% v/v acetic acid
    Adjust pH to 5.5 after dissolving
    Total volume 6mL
1 mg/mL TPP

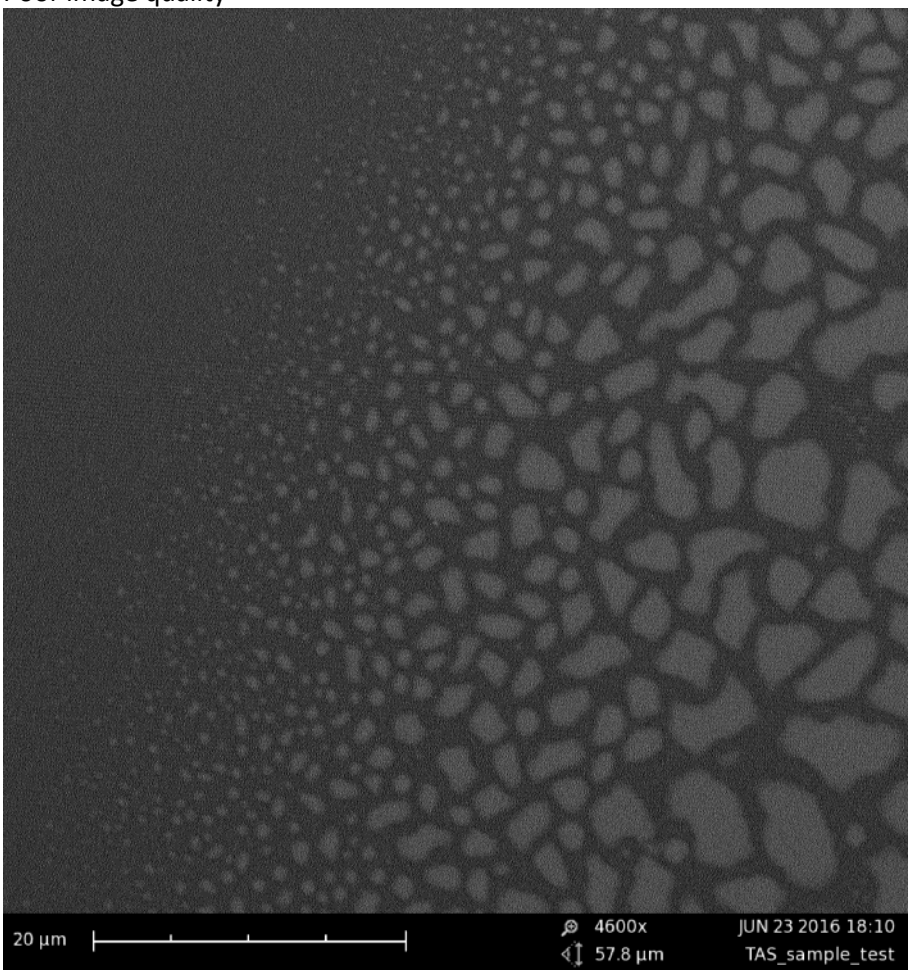
1. 0.1mL chitosan, 0.06mL TPP (adjust both parts to 0.6 mL before mixing)
2. 0.1mL chitosan, 0.1 mL TPP
3. 0.6mL chitosan, 0.36 mL TPP
4. 0.6mL chitosan, 0.6 mL TPP

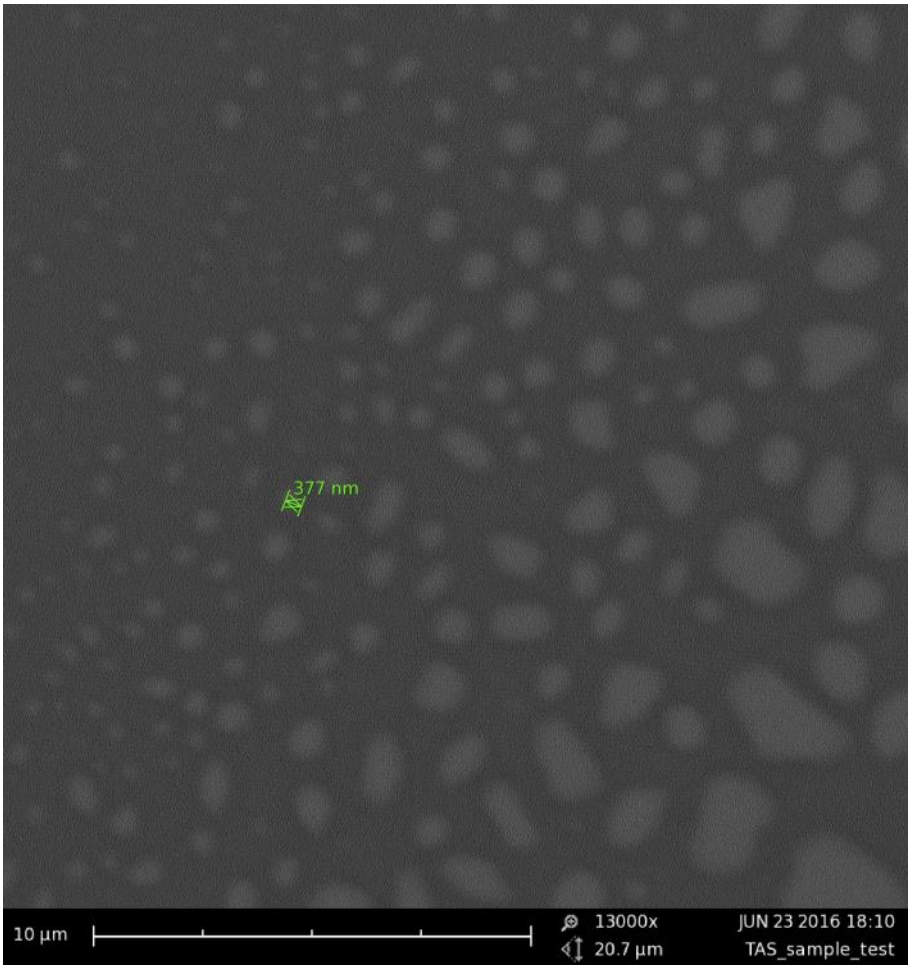
Centrifuge for 30 min at 13000g, room temperature

Dry the sample at 60C for 1 hr

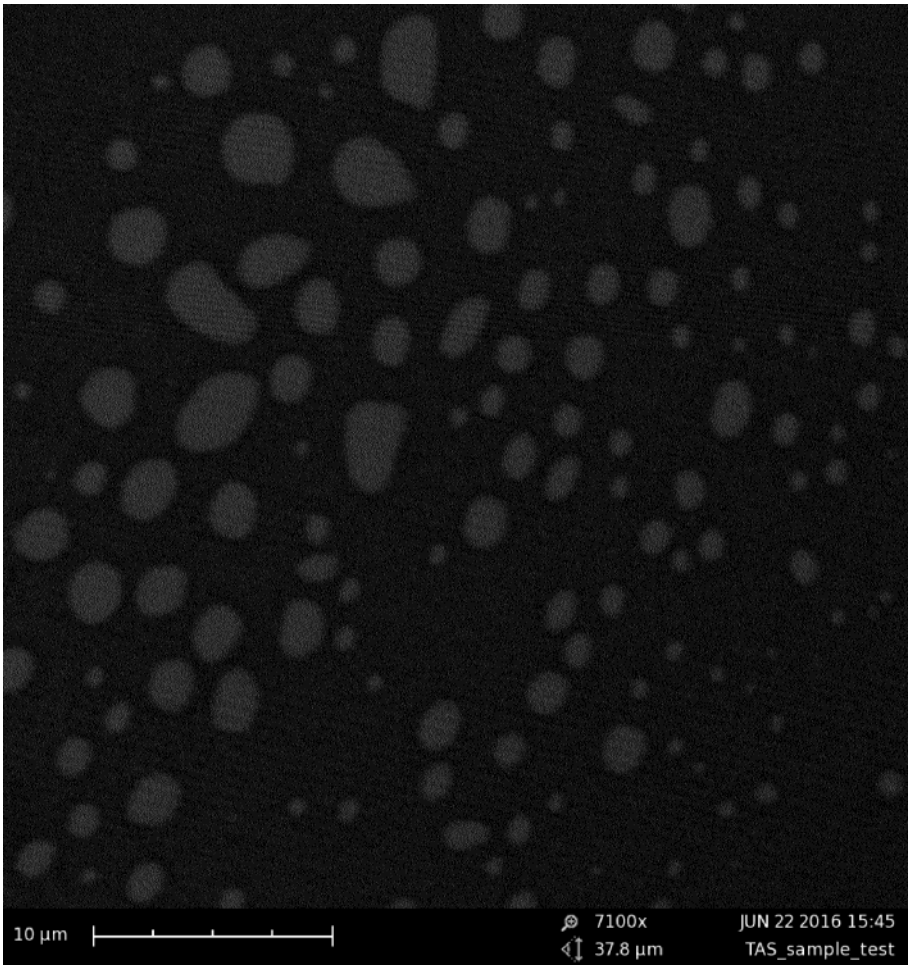
Group one was not evaluated, no precipitate is visible
Same for group 2

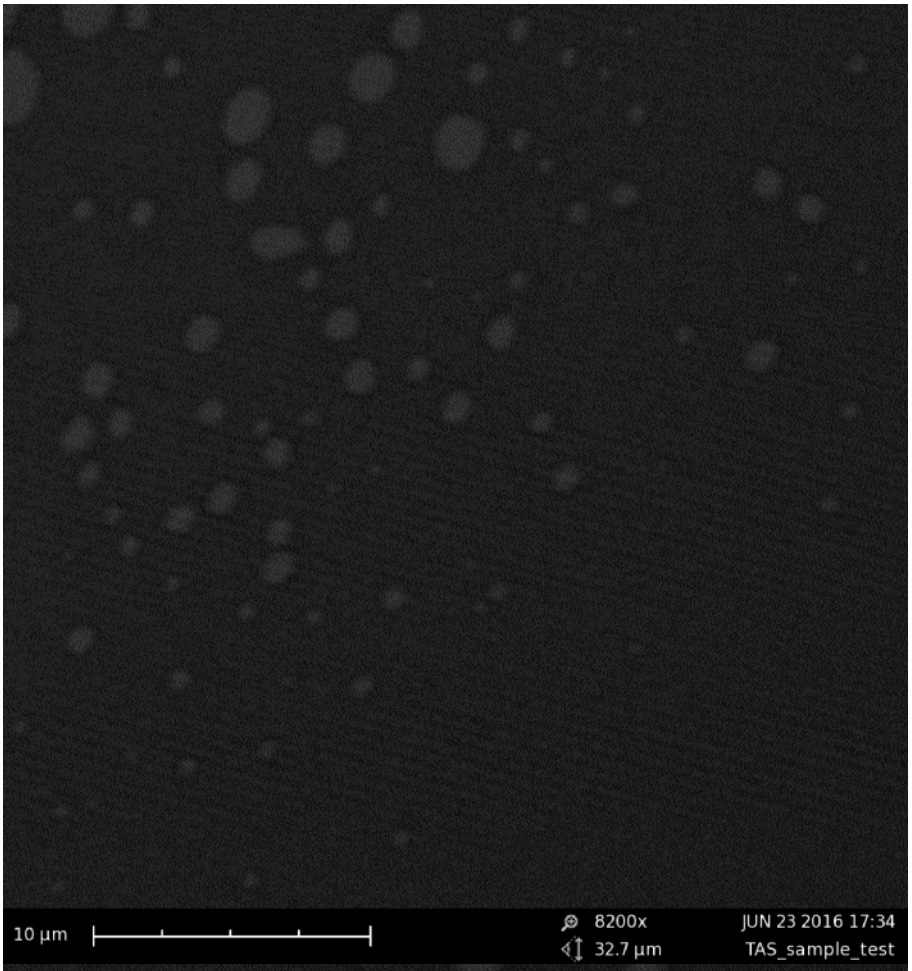
Group 3 has small piece of gel-like precipitate
Poor image quality

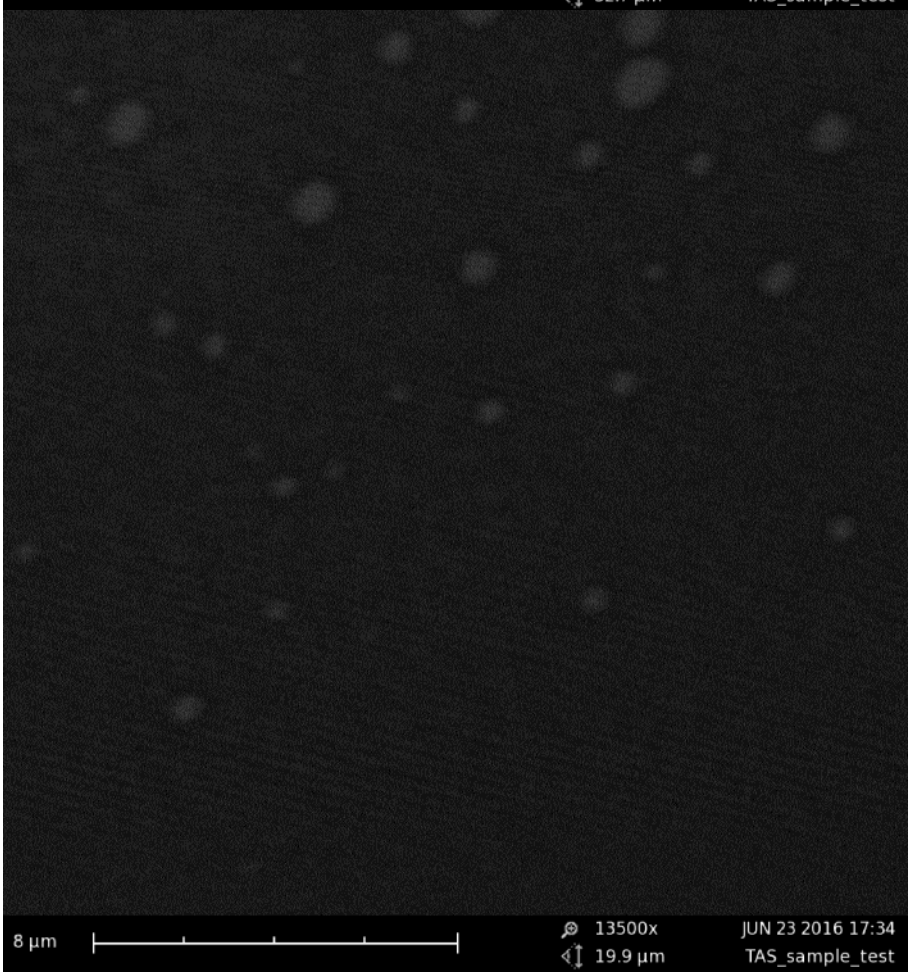


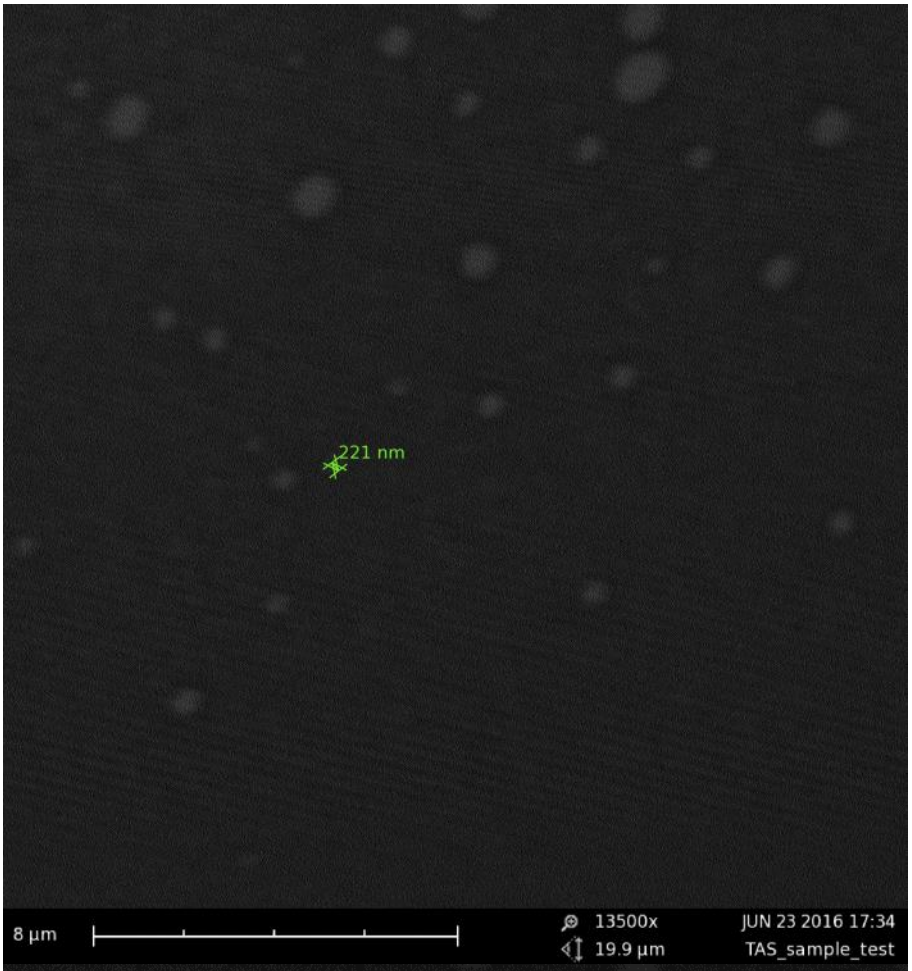


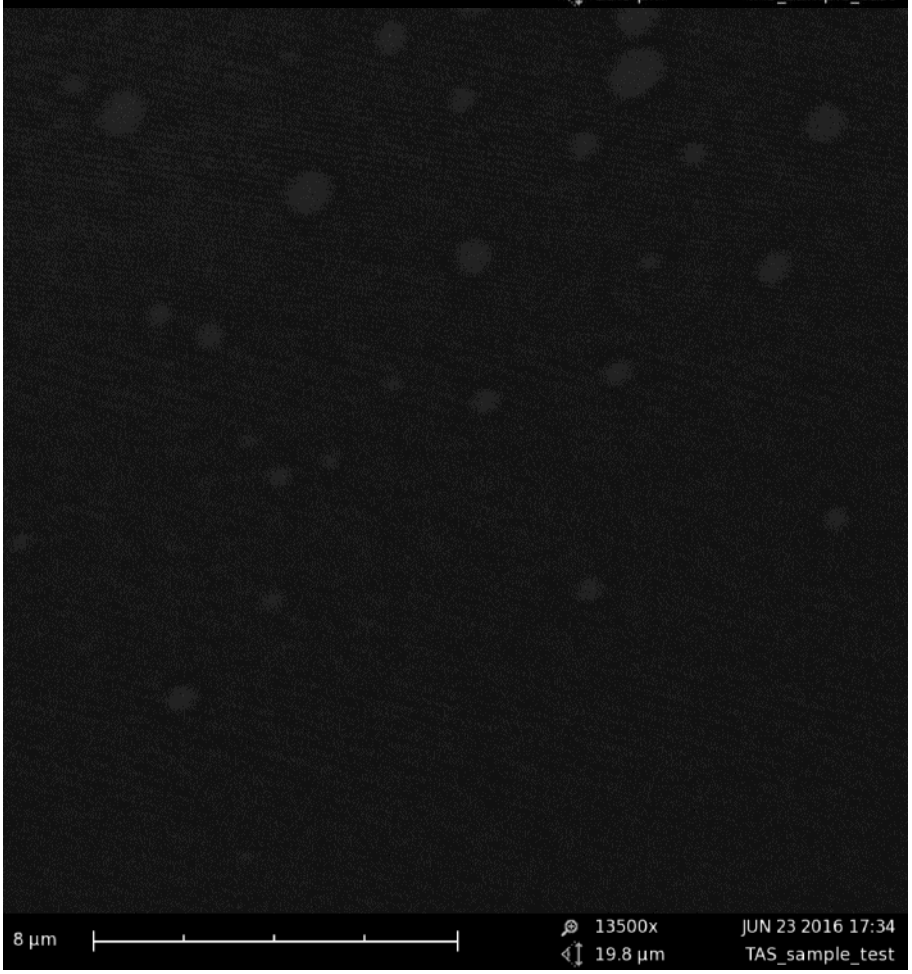
Group 4 has larger piece of precipitate, similar image to group 3

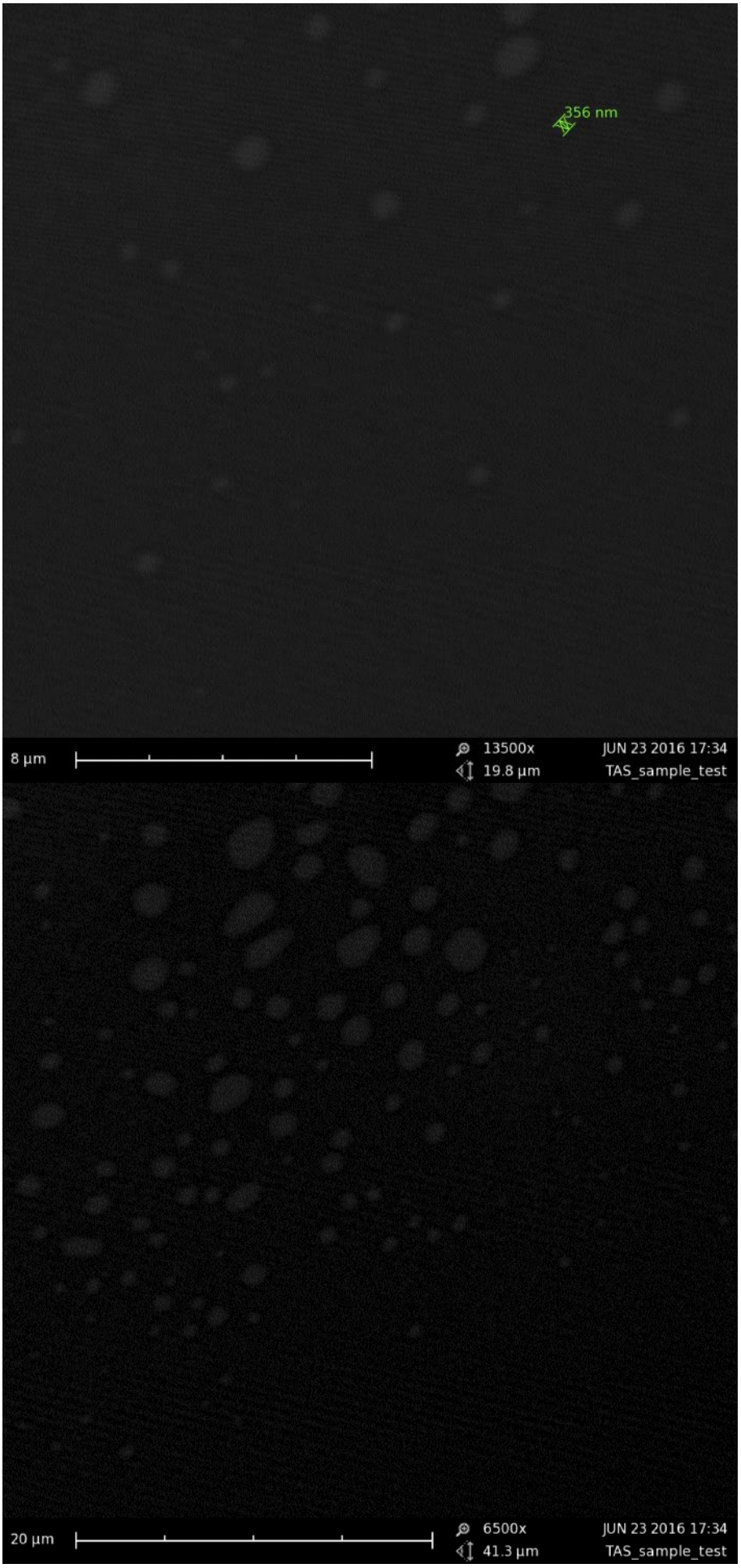

10 µm
7100x
37.8 µm
JUN 22 2016 15:45
TAS_sample_test


10 µm
8200x
32.7 µm
JUN 23 2016 17:34
TAS_sample_test


8 µm
13500x
19.9 µm
JUN 23 2016 17:34
TAS_sample_test


221 nm
8 µm
13500x
19.9 µm
JUN 23 2016 17:34
TAS_sample_test


8 µm
13500x
19.8 µm
JUN 23 2016 17:34
TAS_sample_test


356 nm
8 µm
13500x
19.8 µm
JUN 23 2016 17:34
TAS_sample_test
20 µm
6500x
41.3 µm
JUN 23 2016 17:34
TAS_sample_test

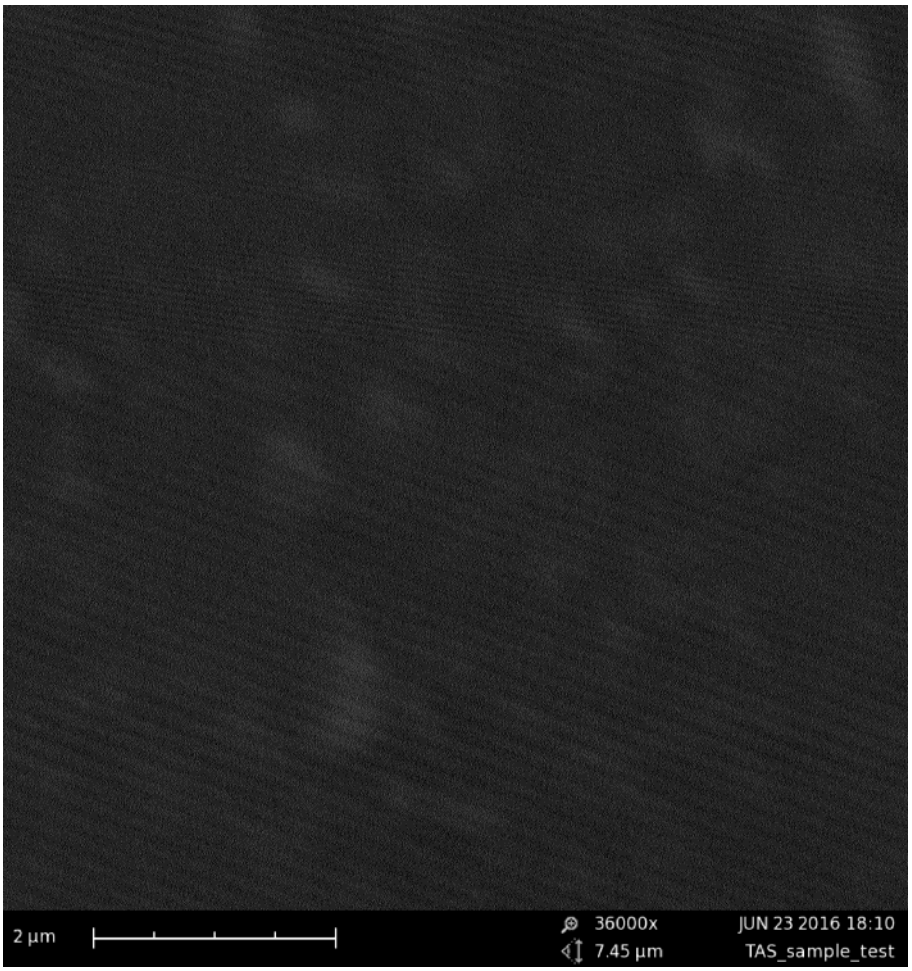
6/27 Nanoparticles made in 6/23 scanned under SEM

2016年6月27日 下午 06:30

Group 3 and Group 4 from 6/23 are repeated 2x

3-1
Poor image – cannot see anything
3-2: Dry by adding 0.01mL of 95% ethanol, 5 times

4-1



4-2: Dry by adding 0.01 mL of 95% ethanol, 5 times

Conclusion:

Poor image quality

6/28 Protein Conditioning Test w/ BSA

Saturday, July 02, 2016 9:39 AM

Goal: To find out to what extent the protein, BSA can remain stable under different thermal, kinetic conditions

Procedure:

Prepare Four 9mg of BSA in 1ml Aqueous Solution and apply different test to each

1) Incubate at Room Temperature for 1 hour
2) Vortex for 1min and incubate at Room Temperature
3) Incubate at 37C for 1 hour
4) Dissolve the protein in 1ml of PH 2 Solution

Result:

Top: Protein stored at 37C for an hour
Bottom: 1ml ddH20

Top: Vortexed Protein
Middle: Protein incubated at Room Temperature
Bottom: 1ml ddH20

Top: Protein in pH2 (for about 5 minutes)
Bottom: 1ml ddH20

Conclusion:

BSA need not be dealt with that much care while making protein encapsulated nanoparticles within 1 hour period. When dissolving the nanoparticles in pH2 it can only be stored for about 20 minutes because after 20 minutes the protein dissolved in pH2 degraded and the color of the solution turned brown.

6/28 Protein Encapsulation Testing w/ BSA 1

Saturday, July 02, 2016 9:37 AM

Goal: To find out whether protein can be encapsulated in our Chitosan Nanoparticles and if they do, with what percent yield

Procedure:

1) Make 1ml of 3mg/ml Chitosan solution with PH5.5 Acetic Acid + NaOH Solution. Vortex to dissolve the Chitosan in the PH 5.5 Solution
2) Make 400ul of 1mg/ml TPP solution and add 10mg of Albumin into the solution. Swirl the solution to fully dissolve TPP
3) Flush Mix Chitosan Solution into the TPP solution with 25ul/50ul of volume each time
4) Create a **negative control** nanoparticle following the same instruction as above but with no protein
5) Centrifuge the flush mixed solution in 13.3 RPM for 50 Minutes at 4 degrees Celsius
6) Store the Nanoparticle w/ Supernatant at -20C or carry on with the Protein Tests
7) Gently Pipet the supernatant of both solutions (Negative and Positive Controls) without disturbing the nanoparticle pellet.
8) Take 50ul of supernatant from both solutions and store them in different 1.5ml micro-centrifuge tubes
9) With the remaining supernatant do a Biuret Test
10) Pour out the remaining supernatant so that you just have the pellets.
11) If Biuret test was successful, blank nanodrop (Protein A280) with 50ul of negative control supernatant and measure the protein concentration of the other several times until you get consistent concentration value.
12) Calculate/Estimate the Percent Yield of the protein in the nanoparticles using the protein concentration in the supernatant.

Result:

Supernatant Biuret Testing

Above: BSA Protein nanoparticle supernatant
Below: Negative Control

Protein Concentration in the Supernatant: 6.8mg/ml
Percent yield of BSA in nanoparticles theoretically: 100% - (6.8/10.1)*100 = 33%

Reference Research Paper:

Conclusion:

More elaborate method to calculate the protein encapsulation needed.

6/28 Protein Encapsulation Test w/ BSA 2

Saturday, July 02, 2016 9:53 AM

Goal: To directly find out the protein concentration in the nanoparticle pellet

Procedure:

Refer to the protocol of Protein Encapsulation Test w/ BSA 1

1) Dissolve the pellet left from the Test 1 in pH 2.

Result:

Biuret Test Failed for the dissolved pellet.
Nanodrop concentrations were all 0 or negative values.

Conclusion:

Ninhydride test should be carried out next time to see if the biuret test failed because of the degradation of protein or was not even encapsulated in the first place.

09/01 Protein Encapsulation Test

2016年8月31日　　下午 02:10

Goal: to observe encapsulation above 33%

Procedure:

Chitosan Solution - 2ml

1. Add 3mg/ml CS to 1% v/v glacial acetic acid
2. Add 90ul/ml 1M NaOH (to raise pH to 5.5)
3. Add 2mg/ml protein

TPP Solution - 2ml

4. Add 1mg/ml TPP
5. Flush mix 50ul CS to TPP and mix with magnetic stirring
6. Centrifuge @ 13 rpm, 4°C, 45 min

7. Negative control was created without the addition of BSA

Results:

To-Do

2016年8月25日　　下午 12:46

Pre-Synthesis To Do List

- [x] Test how much 1M NaOH neutralizes 1% v/v acetic acid (of a 5 ml CS solution)
    - Use 5 drops methyl red indicator to achieve pH app. 4-5
    - Est. Fixed vol. + coc. of NaOH to be added for future np procedures
    - 450ul for 5ml 1% Chitosan solution
- [x] Confer to set final np method (see first page)

Testing NaOH—drop vs dump

2016年9月3日　　下午 02:56

Goal: To investigate how dumping 1M NaOH all at once causes precipitation of Chitosan and have pH increase to 11 when the same amount of 1M NaOH added dropwise do not and maintain pH at 5.5.

Prepare sample:

- 0.2% Acetic acid
- 1.8mg/mL Chitosan
- 165uL 1M NaOH

Total: 40mL

8 tubes

Drop:

- pH probe before/after
- Methyl red before/after

Dump:

- pH probe before/after
- Methyl red before/after

Prepared Sample: 0.2% Acetic acid 1.8mg/mL Chitosan 1M NaOH		
Dripping vs dumping NaOH (5mL chitosan solution)		
	Methyl Red	pH probe
Drip (5 x 20 uL)	pinkish yellow	5.3
4 x 20	pinkish yellow	5
Dump (100uL)	pinkish yellow	5.2
^ we used those solutions to make nanoparticles... didn't succeed		

Dripping or dumping 1M NaOH to alter the pH of the Chitosan solution did not have much difference.

Finalized Nanoparticle Procedure

Tuesday, October 18, 2016 6:07 PM

Refer to Experiment Protocol

Nanoparticle BSA Release V.1

Sunday, August 28, 2016 7:00 PM

Goal: To find out the release and denaturation rate of the BSA encapsulated in Nanoparticles under various solutions (PBS, Tris,Saline and ddH2O)

Procedure:

Prepare 8 1ml Nanoparticle solution. Four containing the protein and the other not containing the protein. (Chitosan, TPP: amount according to the modified nanoparticles procedure and BSA: enough so that protein release is detectable)

Centrifuge the solution at 13.3 rpm or 17.0 xg for 45 minutes.

Take out the supernatant and separately measure the concentration of the protein in the supernatant and with that value estimate amount of protein encapsulated. Throw away the supernatant

Move all 8 nanoparticle pellets into a separate 15 ml tubes.

Put in PBS, Tris, Saline and ddH2O into each tube. One for the nanoparticles with protein and one without.

As soon as the solution is put in time = 0 for protein release.

Every hour or two, measure the absorbance of the new supernatant of the nanoparticles with the protein. Blank with the new supernatant of the nanoparticles without the protein.

Record every single measurement down and note any physical changes in the supernatant or the nanoparticle pellet every time you measure the absorbance.

To be carried out after the Nanoparticle protocol has been solidified :) <3

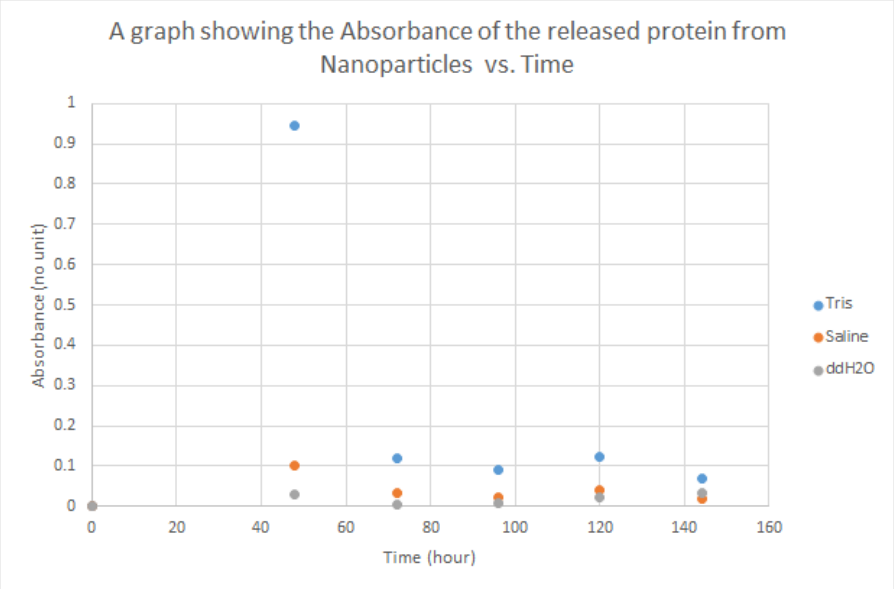
Observation

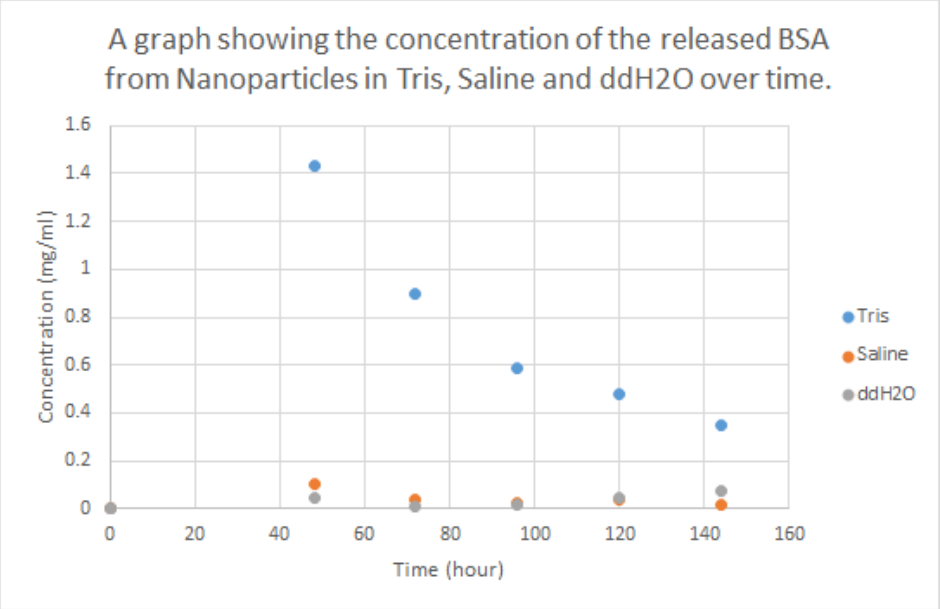
Time	Abs. Tris	Concentration	Abs. Saline	Concentration	Abs. DdH2O	Concentration
0	0		0		0	
24 hr	0.946		0.102		0.031	
48 hr	0.121		0.035		0.006	
72 hr	0.090		0.022		0.009	
96 hr	0.123		0.039		0.022	
120 hr	0.070		0.020		0.035	
144 hr						

Standard curve

BSA Concentration (mg/ml)	Amount of BSA	Tris - Absorbance (280nm)	Saline Absorbance (280nm)	DdH20 Abs. (280nm)	PBS Abs. (280nm)
0.1	0.2	0.267	0.164	0.080	
0.2	0.4	(0.5) 0.457	0.150	0.223	
0.5	1.0	0.655	(1.1) 0.197	(1.1) 0.784	
1.0	2.0	(1.9) 0.943	(1.9) 0.433	1.284 (2.0)+- 0.1	
2.0	4.0	1.799	(4.2) 1.116	2.161	

Result





The concentration was obtained by relating the absorbance data to concentration in the standard curve.

Conclusion:

Regardless of the solution the BSA is released into, there is maximum net amount of protein release around 48 hours.

After 48 hours, the protein concentration decreases for all three solutions. This is high likely to be caused by the denaturation of proteins due to its presence in 37C over time.

Some data points that go out of trend can be explained by the degradation of the nanoparticles, meaning greater release rate of proteins.

The data point of 24th hour is missing so we plan on re-doing this experiment and measure the absorbance at 24th hour to see whether the highest absorbance peak stays at 48th hour or is supposed to be at 24th hour. With the 24th hour data point we can determine whether the protein starts to degrade after 24 hours in those solutions or after 48 hours.

Final Nanoparticle Protein Release V.2 test 4C and 37C

Monday, October 3, 2016 17:59

We used Bradford Assay to measure protein release at 24, 48, and 72 hours
For measurement, we first centrifuged the nanoparticles suspension
We took 40 uL of the supernatant (40 uL of PBS as blank) and added 2 mL Coomassie Brilliant Blue
After 5 minutes, we measured the absorbance at 595nm

We assumed that the initial absorbance is 0
The absorbance values and protein concentration have a linear relationship
We are only looking for the trend, so we did not convert absorbance to concentration

Absorbances at 595nm

	-20 DegresNC	SE	-20 DegresBSA	SE	4 DegresNC	SE	4 DegresBSA	SE	37 DegresNC	SE	37 DegresBSA	SE
Initial	0.01		0.032		0.006		0.033		0.008		0.035	
24hr	0		0.008		0.007		0.013		0.006		0.05	
	-0.001		0.002		0.011		0.007		0.004		0.053	
	-0.0005	0.0005	**0.005**	0.003	**0.009**		**0.01**	0.003	**0.005**		**0.0515**	0.0015
48hr	0.001		0.017		0.004		0.017		0.006		0.056	
	0.004		0.013		0.009		0.012		0.007		0.055	
	0.0025	0.0015	**0.015**	0.002	**0.0065**		**0.0145**	0.0025	**0.0065**		**0.0555**	0.0005
72hr	-0.003		0.006		0.001		0.008		0.01		0.071	
	0		0.009		-0.003		0.007		0.006		0.07	
	-0.0015	0.0015	**0.0075**	0.0015	**-0.001**		**0.0075**	0.0005	**0.008**		**0.0705**	0.000

Protein Encapsulation Tests 09/14 - 09/30

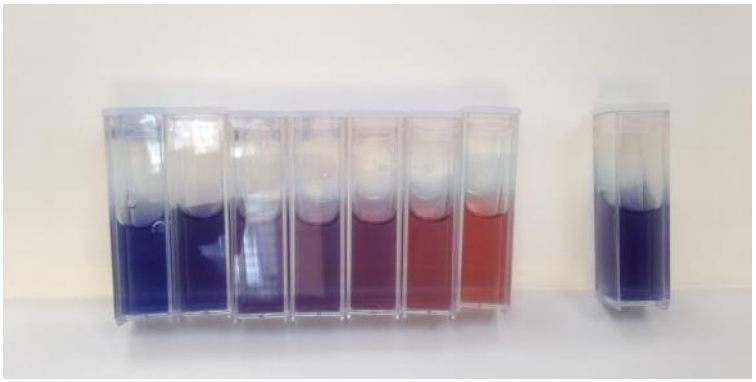
2016年9月21日　　下午 01:56

Test protein concentration within supernatant of BSA loaded nanoparticles using a Bradford Assay.

Known solutions to create the standard curve included

- 40ul of supernatant sample
- 2, 1.5, 1, 0.75, 0.5, 0.25, 0 mg/ml of BSA respectively
- 2 ml of coomassie blue dye

Measure absorbance at 595nm



Reference protocol: http://www.bio-rad.com/LifeScience/pdf/Bulletin_9004.pdf

TPP --> CS NP

- Trial 1: 75% encapsulation

Protein Concentration	Absorbance
2	0.832
1.5	0.727
1	0.651
0.75	0.559
0.5	0.45
0.25	0.318
0	0.173
0.458	**0.424**

- Trial 2: 75% encapsulation

Protein Concentration	Absorbance
0	0
0.25	0.151
0.5	0.255
0.75	0.314
1	0.453
1.5	0.577

CS --> TPP NP

- Trial 1: 28% encapsulation

Protein Concentration	Absorbance
2	0.649
1.5	0.588
1	0.453
0.75	0.36
0.5	0.262
0.25	0.113
0	0
1.33	**0.547**

- Trial 2: 79% encapsulation

Protein Concentration	Absorbance
0	0.004
0.25	0.102
0.5	0.305
0.75	0.37
1	0.464

○

0.75	0.314
1	0.453
1.5	0.577
2	0.667
0.49	**0.243**

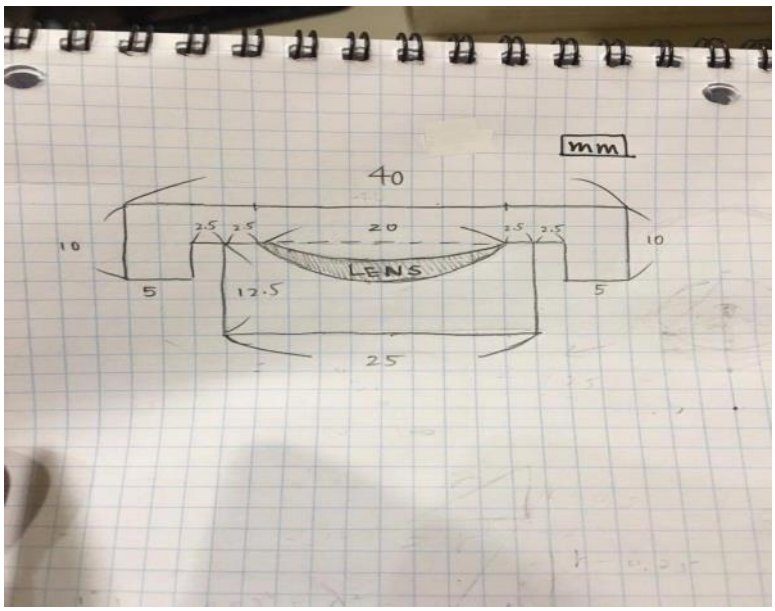
○

0.75	0.37
1	0.464
1.5	0.56
2	0.656
0.41	**0.219**

9/30 Lens Mold

2016年10月18日　　上午 09:47

3D paper design

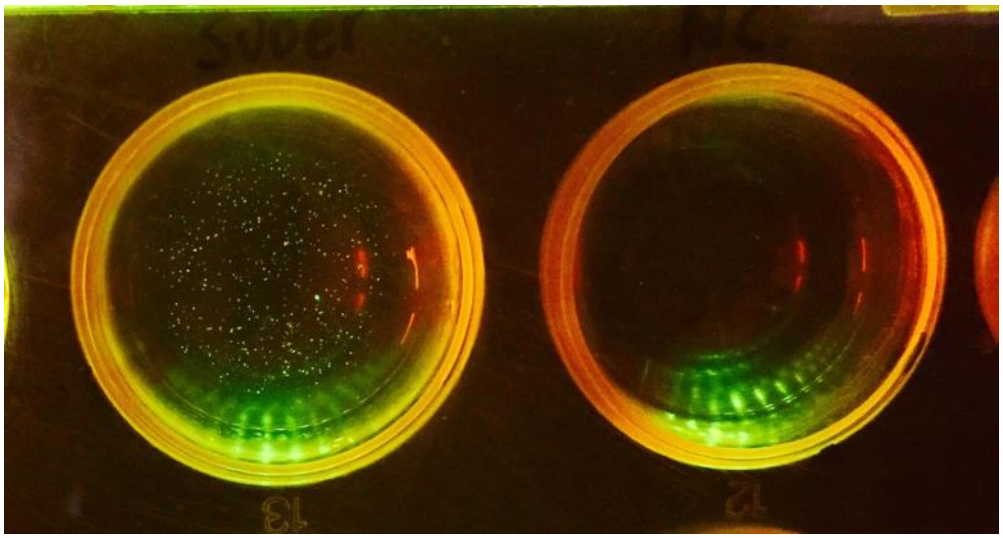


Hydrogel Trials 1, 2, 3

2016年10月18日　　下午 01:56

Trial 1. lens made without the mould, surface was rough, too thick, no curvature

Trial 2. made new contact lenses with the lens mold (left) Surface less rough, made with a mould but still too thick (right)



Trial 3. GfP-containing nanoparticles embedded contact lenses (left) and another one without (right)